Tetrahedron Letters 49 (2008) 7042–7045

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis and structure revision of tyroscherin, a growth inhibitor of IGF-1-dependent tumor cells

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ARTICLE INFO

Article history:
Received 5 September 2008
Revised 20 September 2008
Accepted 25 September 2008
Available online 30 September 2008

Keywords:
Tyroscherin
Inhibitor of IGF-1-dependent growth
Structure revision
One-pot Julia coupling

ABSTRACT

Synthesis of the proposed structure of tyroscherin, a growth inhibitor of IGF-1-dependent cancer cells, was succeeded by one-pot Julia coupling. However, spectral data of the synthetic compound were not identical with those of natural tyroscherin. The stereochemistry of tyroscherin was revised to be 2*S*,3*R*,8*R*,10*R* by syntheses of stereoisomers.

Recently, mechanism-based drugs have been remarkably noticed, since they will potentially provide selective treatments for various diseases such as infections and cancers. Insulin-like growth factor (IGF) plays a key role in human cancer progression,[1] and selective inhibitors of its signal transduction are thought to provide a selective treatment against IGF-dependent tumor cells. In 2004, Hayakawa et al. isolated tyroscherin from the mycelium of *Pseudallescheria* sp. as a potent and selective inhibitor of IGF-1-dependent growth of MCF-7 human breast cancer cell.[2] We started the synthesis of tyroscherin with the intention of further research on its biological activity and structure–activity relationship.

Herein, we report the synthesis of the proposed structure of tyroscherin (**1**). However, spectral data of synthetic **1** were not identical with those of natural tyroscherin. The stereochemistry of tyroscherin is revised to be 2*S*,3*R*,8*R*,10*R*, as shown in Figure 1, by syntheses of stereoisomers.

The synthesis of the proposed structure (**1**) is shown in Scheme 1. D-Tyrosine was protected in a usual manner to give ester **3**. C_3 elongation of ester **3** via the corresponding Weinreb amide afforded amino ketone **4**. This ketone was subjected to stereoselective reduction[3] to give *syn*-amino alcohol **5**, whose stereochemistry was confirmed by NOE experiment after conversion into cyclic carbamate **11**. After protection and deprotection, *syn*-amino alcohol **5** was converted to the PT-sulfone **7** under Mitsunobu condition.[4] Then one-pot Julia coupling[5,6] of sulfone **7** and known aldehyde **8**[7] gave (*E*)-olefin **9** selectively. Though N-methylation of the compound **9** did not proceed directly, it was succeeded in good yield after replacement of the protecting group. Finally, deprotection of **10** gave desired compound, the proposed structure of tyroscherin (**1**). However, the ^{1}H NMR spectral data of the synthetic compound **1**[8] were not identical with those reported for natural tyroscherin.[2] Chemical shifts of 1-H, 2-H, and 3-H were much different between natural tyroscherin and synthetic **1** as shown in Table 1. Hayakawa et al. have determined the relative configuration at C-2 and C-3 by analysis of ^{1}H–^{1}H and ^{1}H–^{13}C coupling constants,[2,9] after determination of the absolute configuration at C-3 by modified Mosher method.[10] We supposed the correct relative stereochemistry of natural compound to be 2,3-*anti*. To ascertain the correct structure of natural tyroscherin, we started synthesis of 2,3-*anti*-stereoisomers of **1**.

Synthesis of 2,3-*anti*-isomers is shown in Scheme 2. Ester **12**, derived from L-tyrosine, was subjected to N-methylation using NaH and was subsequently converted to Weinreb amide **13**. During this sequence, partial racemization was observed, and enantiomeric purity of **13** was determined to be 33% ee by chiral HPLC (Chiralcel OD, hex/*i*-PrOH = 19:1). This compound was reduced to aldehyde, which was reacted with siloxypropyllithium to afford a mixture of *anti*- and *syn*-amino alcohols. After protection of

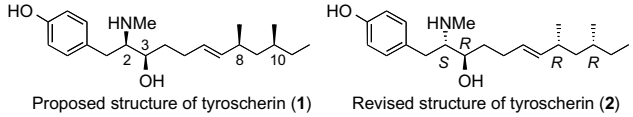

Figure 1. Proposed and revised structures of tyroscherin.

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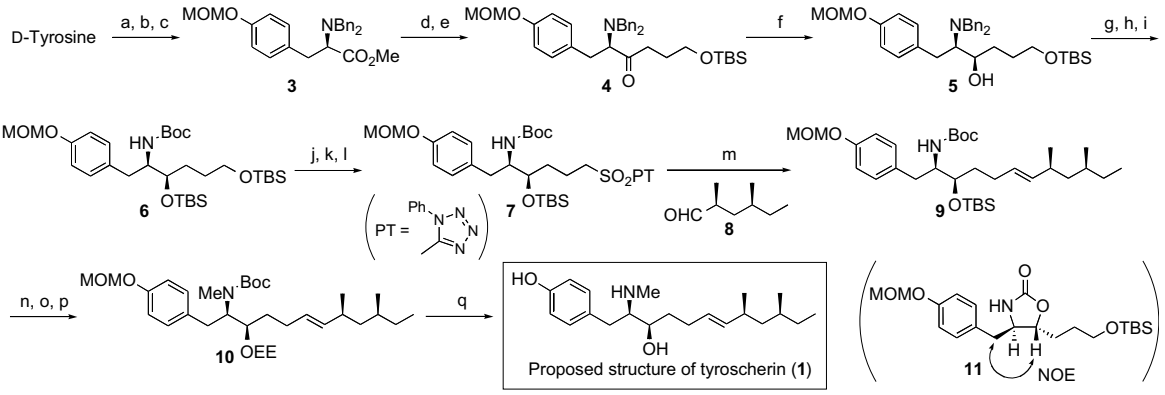
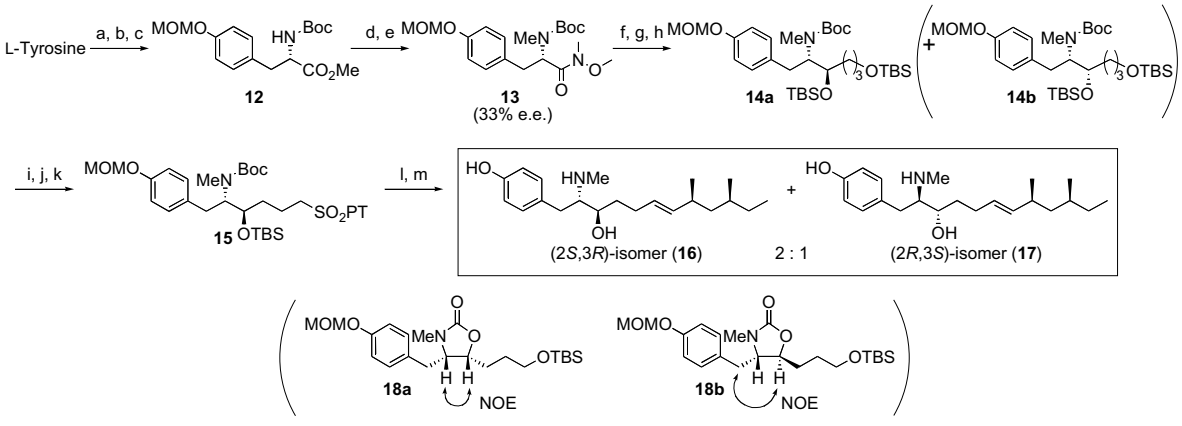
doi:10.1016/j.tetlet.2008.09.141



Scheme 1. Synthesis of the proposed structure of tyroscherin (**1**). Reagents and conditions: (a) $SOCl_2$, MeOH, reflux, quant.; (b) BnBr, DIPEA, DMF, 0 °C to rt, 97%; (c) MOMCl, K_2CO_3, CH_3CN, 0 °C to rt, 95%; (d) MeNHOMe·HCl, *i*-PrMgBr, THF, −20 °C to rt, 93%; (e) $TBSO(CH_2)_3I$, *t*-BuLi, ether, −78 °C to rt, 86%; (f) $NaBH_4$, MeOH, EtOH, −20 °C, 99%, single isomer; (g) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 78%; (h) H_2, Pd–C, EtOH, EtOAc, rt, 92%; (i) $(Boc)_2O$, THF, rt, 94%; (j) Dowex-50, MeOH, rt, 71% (and 25% of diol); (k) PTSH, DEAD, PPh_3, THF, 0 °C to rt, 99%; (l) H_2O_2, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, EtOH, 0 °C to rt, 85%; (m) KHMDS, THF, −78 °C to rt, 70% (based on recovery); (n) Dowex-50, MeOH, rt, 96%; (o) $CH_2{=}CHOEt$, PPTS, CH_2Cl_2, rt, quant.; (p) NaH, MeI, THF, reflux, quant.; (q) HCl, MeOH, rt, 99%.

Table 1
Selected ^1H NMR (500 MHz, $CDCl_3$) data and specific rotations for natural tyroscherin and synthetic stereoisomers

	^1H NMR (ppm, multiplicity, Hz)							$[\alpha]_D$ (*c* 0.35, MeOH)
	1-H_a	1-H_b	2-H	3-H	12-H	8-Me	10-Me	
Natural tyroscherin[1]	2.86 dd, 14.0, 7.0	2.91 dd, 14.0, 7.0	3.34 ddd, 7.0, 7.0, 3.0	3.83 dt, 10.0, 3.0	0.84 t, 7.0	0.91 d, 6.5	0.82 d, 6.5	−21
Proposed structure (**1**)	2.87 dd, 13.8, 8.3	**2.96** dd, 13.8,6.0	**3.22** ddd, 8.3,6.0, 4.7	**3.62** ddd, 6.8, 5.3,4.7	0.85 t, 7.0	0.89 d, 6.2	0.81 d, 6.5	+11
Mixture of *anti*-isomers (Scheme 2) Major (**16**)	2.86 dd, 14.7, 7.9	**2.93** dd, 14.7, 6.7	3.32–3.38 m, overlapped	3.81–3.87 m, overlapped	0.84 t, 7.3	**0.92** d, 6.8	**0.81** d, 6.5	—
Minor (**17**)	2.86 dd, 14.7, 7.9	2.91 dd, 14.7,7.0	3.32–3.38 m, overlapped	3.81–3.87 m, overlapped	0.84 t, 7.3	0.91 d, 6.8	0.82 d, 6.5	—
(2*R*,3*S*,8*S*,10*S*)-isomer (**17**)	2.86 dd, 14.7, 7.9	2.91 dd, 14.7, 7.0	3.34 ddd, 7.9, 7.0, 3.0	3.83 ddd, 9.4, 3.6, 3.0	0.84 t, 7.3	0.91 d, 6.8	0.82 d, 6.5	+20



Scheme 2. Synthesis of mixture of (2*S*,3*R*)-isomer (**16**) and (2*R*,3*S*)-isomer (**17**). Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) NaOH, H_2O, then $(Boc)_2O$, 0 °C, THF; (c) MOMCl, DIPEA, CH_2Cl_2, 0 °C to rt, 99% in three steps; (d) NaH, MeI, THF, reflux; (e) MeNHOMe·HCl, *i*-PrMgBr, THF, −20 °C to rt, 83% in two steps; (f) DIBAL, Et_2O, 0 °C, 89%; (g) $TBSO(CH_2)_3I$, *t*-BuLi, Et_2O, −78 °C to rt, 79%; (h) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 96%, *dr* = 7:1; (i) Dowex-50, MeOH, H_2O, rt, 97%; (j) DEAD, PPh_3, PTSH, THF, 0 °C to rt; (k) H_2O_2, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, EtOH, 0 °C to rt, 62% in 2 steps; (l) KHMDS, **8**, THF, −78 °C to rt, 39%; (m) TFA, THF, MeOH, H_2O, 50 °C, quant.

hydroxy group, *anti*- and *syn*-isomers were separated by silica gel chromatography (**14a**:**14b** = 7:1). The stereochemistries at C-3 were determined by NOE experiments after conversion to cyclic carbamates **18a** and **18b**, respectively. In a similar manner to the synthesis of **1**, the *anti*-isomer **14a** was converted to inseparable mixture of (2*S*,3*R*)-isomer (**16**) and (2*R*,3*S*)-isomer (**17**) (**16**:**17** = 2:1), which came from low enantiomeric purity of **13** (33% ee). ^1H NMR spectrum of the mixture (**16** and **17**) showed that signals of the major component were not identical with those of natural tyroscherin, while signals of the minor component were observed at quite similar chemical shifts to those of natural tyroscherin[2] (Fig. 2 and Table 1).

Thus, we were confident that the minor component **17** had the same relative configuration as natural tyroscherin. Hayakawa et al.

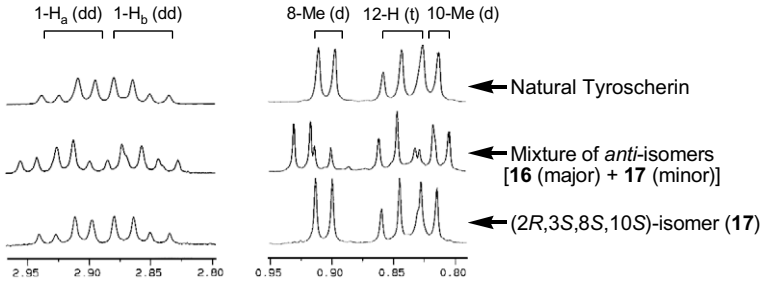
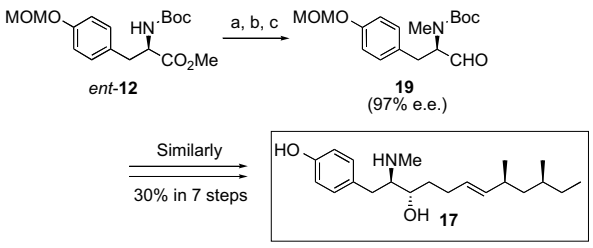


Figure 2. ^{1}H NMR signals of natural and synthesized compounds (1-H and Me groups).



Scheme 3. Synthesis of (2*R*,3*S*,8*S*,10*S*)-isomer (**17**). Reagents and conditions: (a) MeNHOMe · HCl, *i*-PrMgBr, THF, −20 °C to rt, 82%; (b) NaH, MeI, DMF, −20 °C, 76%; (c) DIBAL, ether, 0 °C, 97% ee.

have determined the absolute configuration at C-3 by modified Mosher method, as already mentioned, and the absolute configurations at C-8 and C-10 by degradation studies.[2] Because several fungal metabolites, such as squalestatin S1,[11] TMC-171,[12] and lunatoic acids,[13] have been reported to have similar (*S*,*S*)-dimethylalkyl chain, we assumed that tyroscherin has the same stereochemistries at C-8 and C-10 as these compounds. So, we started the stereoselective synthesis of **17** (Scheme 3). Ester *ent*-**12**, derived from D-tyrosine, was converted to the corresponding Weinreb amide and was subjected to subsequent N-methylation.[14] Consequently, *ent*-**13** was obtained with almost no racemization. Reduction of the amide gave aldehyde **19**, whose enantiomeric purity was determined to be 97% ee by chiral HPLC (Chiralpak AD–H, hex/*i*-PrOH = 19:1). In the same manner as Scheme 2, the aldehyde **19** was transformed into **17**. After recrystallization, (2*R*,3*S*,8*S*,10*S*)-isomer (**17**) was obtained in a diastereomerically pure form. ^{1}H NMR spectrum of **17** was completely identical with that of natural tyroscherin as we expected (Fig. 2 and Table 1), but unfortunately, **17** showed opposite sign of specific rotation to that of natural tyroscherin {**17**: $[\alpha]_D^{24}$ +20 (*c* 0.35, MeOH), natural tyroscherin: $[\alpha]_D^{24}$ −21 (*c* 0.35, MeOH)[2]}. From these results, we finally concluded that the correct stereostructure of natural tyroscherin must be **2**, an enantiomer of **17**.

The stereoselective synthesis of (2*S*,3*R*,8*R*,10*R*)-isomer (**2**) is shown in Scheme 4. Ester **12** was converted to Weinreb amide, and N-methylation was followed by LAH reduction to give aldehyde *ent*-**19**. Enantiomeric purity of *ent*-**19** was determined to be >99% ee by chiral HPLC. Reaction of the aldehyde with siloxypropyllithium in THF was followed by protection as TBS ether to give the *anti*-isomer **14a** (**14a**:**14b** = 13:1, separated by silica gel chromatography). The *anti*-isomer **14a** was converted to the corresponding sulfone **15**, and one-pot Julia coupling of **15** and *ent*-**8** afforded *trans*-olefin **20** selectively. Finally, deprotection of **20** gave **2** successfully as colorless crystals. Its specific rotation was $[\alpha]_D^{25}$ −21 (*c* 0.35, MeOH), and its melting point and spectroscopic data of **2**[15] were fully identical to those of natural tyroscherin.[2] From all of these results, the stereochemistry of natural tyroscherin is determined to be 2*S*,3*R*,8*R*,10*R* as shown in Figure 1.

In summary, we succeeded in the first synthesis of tyroscherin (and its stereoisomers), in 14.4% overall yield from L-tyrosine, and revised the absolute configuration of tyroscherin to be 2*S*,3*R*,8*R*,10*R*. Our work is under way to improve some steps of the synthesis, and to submit these isomers to further biological assay. Results will be reported in a full account.

Acknowledgments

The authors sincerely thank Professor Y. Hayakawa, Tokyo University of Science, for a kind gift of the spectral charts of natural tyroscherin. This work was partly supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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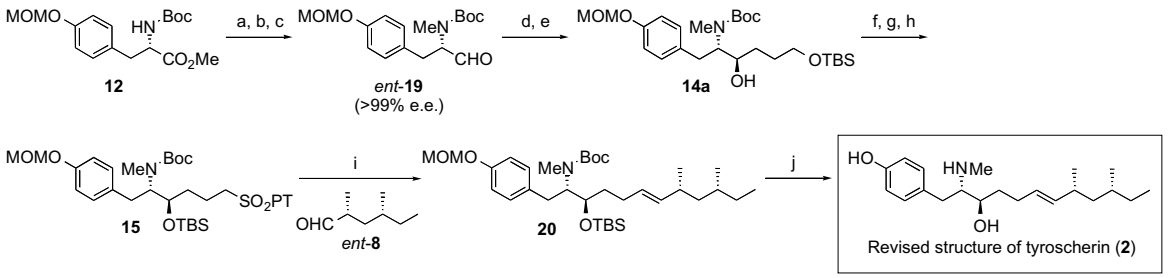

Scheme 4. Synthesis of (2*S*,3*R*,8*R*,10*R*)-isomer (**2**). Reagents and conditions: (a) MeNHOMe·HCl, *i*-PrMgBr, THF, −20 °C to rt, 73%; (b) NaH, MeI, DMF, −20 °C, 95%; (c) $LiAlH_4$, ether, 0 °C, >99% ee; (d) $I(CH_2)_3OTBS$, *t*-BuLi, THF, −78 °C; (e) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 59% in three steps, *dr* = 13:1; (f) Dowex-50, MeOH, H_2O, rt, 82%; (g) PTSH, DEAD, PPh_3, THF, 0 °C to rt; (h) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, H_2O_2, EtOH, 0 °C to rt, 62% in two steps; (i) KHMDS, THF, −78 °C to rt, 70%; (j) TFA, THF, MeOH, H_2O, 50 °C, quant.

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8. *Analytical and spectral data of synthesized* **1**: n_D^{25}1.4714. $[\alpha]_D^{25}$ +11 (*c* 0.35, CH_3OH). ^{1}H NMR (500 MHz, CD_3OD) d (ppm) 0.81 (3H, d, *J* = 6.5 Hz, 10-Me), 0.85 (3H, t, *J* = 7.0 Hz, 12-H), 0.89 (3H, d, *J* = 6.2 Hz, 8-Me), 0.97 (1H, ddd, *J* = 13.0, 8.6, 4.2 Hz, 9-H_a), 1.13 (1H, m, 11-H_a), 1.2-1.35 (2H, m, 10-H, 11-H_b), 1.21 (1H, ddd, *J* = 13.0, 9.4, 4.7 Hz, 9-H_b), 1.5-1.6 (2H, m, 4-H), 1.95–2.15 (3H, m, 5-H, 8-H), 2.67 (3H, s, *N*-Me), 2.87 (1H, dd, *J* = 13.8, 8.3 Hz, 1-H_a), 2.96 (1H, dd, *J* = 13.8, 6.0 Hz, 1-H_b), 3.22 (1H, ddd, *J* = 8.3, 6.0, 4.7 Hz, 2-H), 3.63 (1H, ddd, *J* = 6.8, 5.3, 4.7 Hz, 3-H), 5.16 (1H, dd, *J* = 15.1, 8.1 Hz, 7-H), 5.27 (1H, dt, *J* = 15.1, 6.5 Hz, 6-H), 6.77 (2H, quasi d, *J* = 8.1 Hz, 3′-H, 5′-H), 7.11 (2H, quasi d, *J* = 8.1 Hz, 2′-H, 6′-H). ^{13}C NMR (125 MHz, CD_3OD) δ (ppm) 11.7, 19.3, 22.3, 29.3, 31.1, 31.8, 33.1, 34.1, 35.4, 35.7, 45.5, 65.9, 68.7, 116.8, 127.6, 128.3, 131.4, 138.6, 157.9. IR (KBr) ν = 3382, 2958, 1681, 1517, 1202 cm^{-1}. ESI-TOFMS *m*/*z* calcd for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2741, found 334.2773.
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15. *Analytical and spectral data of synthesized* **2**: mp 122–126 °C. $[\alpha]_D^{25}$ −21 (*c* 0.35, CH_3OH). ^{1}H NMR (500 MHz, CD_3OD) d (ppm) 0.82 (3H, d, *J* = 6.5 Hz, 10-Me), 0.84 (3H, t, *J* = 7.3 Hz, 12-H), 0.91 (3H, d, *J* = 6.8 Hz, 8-Me), 0.99 (1H, ddd, *J* = 13.5, 9.7, 4.8 Hz, 9-H_a), 1.13 (1H, m, 11-H_a), 1.22 (1H, ddd, *J* = 13.5, 9.7, 4.8 Hz, 9-H_b), 1.25–1.35 (2H, m, 10-H, 11-H_b), 1.45–1.6 (2H, m, 4-H), 1.99 (1H, m, 5-H_a), 2.1–2.25 (2H, m, 5-H_b, 8-H), 2.62 (3H, s, *N*-Me), 2.86 (1H, dd, *J* = 14.7, 7.9 Hz, 1-H_a), 2.91 (1H, dd, *J* = 14.7, 7.0 Hz, 1-H_b), 3.34 (1H, ddd, *J* = 7.9, 7.0, 3.0 Hz, 2-H), 3.83 (1H, ddd, *J* = 9.4, 3.6, 3.0 Hz, 3-H), 5.22 (1H, dd, *J* = 15.5, 8.3 Hz, 7-H), 5.33 (1H, dt, *J* = 15.5, 6.7 Hz, 6-H), 6.78 (2H, quasi d, *J* = 8.5 Hz, 3′-H, 5′-H), 7.10 (2H, quasi d, *J* = 8.5 Hz, 2′-H, 6′-H). ^{13}C NMR (125 MHz, CD_3OD) d (ppm) 11.7, 19.4, 22.3, 29.9, 31.1, 32.4, 33.1, 33.2, 35.8, 45.6, 66.8, 68.7, 116.9, 127.6, 128.4, 131.3, 138.8, 158.0. IR (KBr) ν = 3239, 2961, 1671, 1203, 1185, 1146 cm^{-1}. ESI-TOFMS *m*/*z* calcd for $C_{21}H_{36}NO_2$ $[M+H]^+$ 334.2741, found 334.2773.